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10	54	((collagen) near3 (fusion adj protein)) and (E adj coli)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/07/02 15:15
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AU   Yang, Chunlin; Hillas, Patrick J.; Baez, Julio A.; Nokelainen, Minna;
     Balan, Juliana; Tang, James; Spiro, Robert; Polarek, James W.
CS   FibroGen Inc., San Francisco, CA, USA
SO   BioDrugs (2004), 18(2), 103-119
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TI   Coexpression of α and β subunits of prolyl 4-hydroxylase
     stabilizes the triple helix of recombinant human type X collagen
AU   Wagner, Klaus; Poschl, Ernst; Turnay, Javier; Baik, Jeong-Mi;
     Pihlajaniemi, Taina; Frischholz, Svenja; Von der Mark, Klaus
CS   Department of Experimental Medicine I, Nikolaus-Fiebiger Center fur
     Molecular Medicine, University of Erlangen-Nuremberg, Erlangen, D-91054,
     Germany
SO   Biochemical Journal (2000), 352(3), 907-911
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CS Collagen Research Unit, Biocenter and Department of Medical Biochemistry, University of Oulu, Oulu, Finland
SO Matrix Biology (1998), 16(6), 329-338
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TI Assembly of human prolyl 4-hydroxylase and type III collagen in the yeast Pichia pastoris: formation of a stable enzyme tetramer requires coexpression with collagen and assembly of a stable collagen requires coexpression with prolyl 4-hydroxylase
AU Vuorela, Annamari; Myllyharju, Johanna; Nissi, Ritva; Pihlajaniemi, Taina; Kivirikko, Kari I.
CS Collagen Research Unit, Biocenter and Department of Medical Biochemistry, University of Oulu, Oulu, FIN-90220, Finland
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AU Yang C.; Hillas P.J.; Baez J.A.; Nokelainen M.; Balan J.; Tang J.; Spiro R.; Polarek J.W.
CS Dr. J.W. Polarek, FibroGen Inc., 225 Gateway Blvd, South San Francisco, CA 94080, United States. jpolarek@Fibrogen.com
SO BioDrugs, (2004) 18/2 (103-119).
Refs: 183
ISSN: 1173-8804 CODEN: BIDRF4
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DT Journal; General Review
FS 027 Biophysics, Bioengineering and Medical Instrumentation
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TI The application of recombinant human collagen in tissue engineering
AU Yang, Chunlin; Hillas, Patrick J.; Baez, Julio A.; Nokelainen, Minna; Balan, Juliana; Tang, James; Spiro, Robert; Polarek, James W.
CS FibroGen Inc., San Francisco, CA, USA
SO BioDrugs (2004), 18(2), 103-119
CODEN: BIDRF4; ISSN: 1173-8804
PB Adis International Ltd.
DT Journal
LA English
AB Collagen is the main structural protein in vertebrates. It plays an essential role in providing a scaffold for cellular support and thereby affecting cell attachment, migration, proliferation, differentiation, and survival. As such, it also plays an important role in numerous approaches to the engineering of human tissues for medical applications related to tissue, bone, and skin repair and reconstruction. Currently, the collagen used in tissue engineering applications is derived from animal tissues, creating concerns related to the quality, purity, and predictability of its performance. It also carries the risk of transmission of infectious agents and precipitating immunol. reactions. The recent development of recombinant sources of human collagen provides a reliable, predictable and chemical defined source of purified human collagens that is free of animal components. The triple-helical collagens made by recombinant technol. have the same amino acid sequence as human tissue-derived collagen. Furthermore, by achieving the equivalent extent of proline hydroxylation via **coexpression** of genes encoding **prolyl hydroxylase** with the **collagen** genes, one can produce collagens with a similar degree of stability as naturally occurring material. The recombinant production process of collagen involves the generation of single triple-helical mols. that are then used to construct more complex three-dimensional structures. If one loosely defines tissue engineering as the use of a biocompatible scaffold combined with a biol. active agent (be it a gene or gene construct, growth factor or other biol. active agent) to induce tissue regeneration, then the production of recombinant human collagen enables the engineering of human tissue based on a human matrix or scaffold. Recombinant human collagens are an efficient scaffold for bone repair when combined with a recombinant bone morphogenetic protein in a porous, sponge-like format, and when presented as a membrane, sponge or gel can serve as a basis for the engineering of skin, cartilage and periodontal, ligament, depending on the specific requirements of the chosen application.
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AN 2001:48089 CAPLUS
DN 134:248503
TI Coexpression of α and β subunits of prolyl 4-hydroxylase stabilizes the triple helix of recombinant human type X collagen
AU Wagner, Klaus; Poschl, Ernst; Turnay, Javier; Baik, Jeong-Mi; Pihlajaniemi, Taina; Frischholz, Svenja; Von der Mark, Klaus
CS Department of Experimental Medicine I, Nikolaus-Fiebiger Center fur Molecular Medicine, University of Erlangen-Nuremberg, Erlangen, D-91054, Germany
SO Biochemical Journal (2000), 352(3), 907-911
CODEN: BIJOAK; ISSN: 0264-6021
PB Portland Press Ltd.
DT Journal
LA English

AB The authors have reported previously on the expression of recombinant human type X collagen (hrColX) in HEK 293 and HT 1080 cells by using the eukaryotic expression vector pCMVsis (in which CMV stands for cytomegalovirus). Several stably transfected clones secreted full-length triple-helical hrColX mols. in large amts., but the secreted collagen was underhydroxylated, with a hydroxyproline-to-proline ratio of 0.25 and a melting temperature (Tm) of 31°. By comparison, native chicken type X procollagen has a Tm of 46°. To stabilize the triple helix of hrColX, an hrColX-expressing clone (A6/16) was co-transfected with both α and β subunits of human prolyl 4-hydroxylase. Clones were selected that secreted proα1(X) collagen chains with an apparent mol. mass of 75 kDa and an increased hydroxyproline-to-proline ratio of close to 0.5. As a result of enhanced prolyl hydroxylation, the Tm of the hrColX was increased to 41° as measured by CD anal. at various temps. The CD spectra indicated a min. ellipticity at 198 nm and a peak at 225 nm at 20°, confirming the presence of a triple helix. The same Tm of 41° was measured for the triple-helical core fragments of hrColX of 60-65 kDa that were retained after brief digestion with chymotrypsin/trypsin at increasing temps. This shows that the human cell line HEK-293 is suitable for the simultaneous expression of three genes and the stable production of substantial amts. of recombinant, fully hydroxylated type X collagen over several years.

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DT Journal
LA English
AB Insect cells coinfected with two baculoviruses, one coding for the proα chains of human type II procollagen and the other for both the α and β subunits of human prolyl 4-hydroxylase, produced the cartilage-specific type II collagen with a stable triple helix. The highest expression levels, up to 50 mg/l of type II collagen, were obtained in suspension culture using a modified construct in which sequences coding for the signal peptide and N propeptide of type II procollagen had been replaced by those for type III procollagen. The type III N propeptide artificially generated into type II procollagen was found to be cleaved at a much higher rate than the wild-type type II N propeptide, probably because the former interacted poorly with the triple-helical domain of type II procollagen. The amino acid composition of the recombinant type II collagen was very similar to that of the non-recombinant protein, but the hydroxylysine content was only 17% and that of glycosylated hydroxylysines was equally low. The hydroxylysine content was increased to the level found in the non-recombinant collagen by using an addnl. baculovirus coding for lysyl hydroxylase, and a substantial increase was also found in the glycosylated hydroxylysine content. No difference in thermal stability was found between the low- and high-hydroxylysine collagens.

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ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2004 ACS on STN

AN 1998:24156 CAPLUS
DN 128:164334
TI Assembly of human prolyl 4-hydroxylase and type III collagen in the yeast Pichia pastoris: formation of a stable enzyme tetramer requires coexpression with collagen and assembly of a stable collagen requires coexpression with prolyl 4-hydroxylase
AU Vuorela, Annamari; Myllyharju, Johanna; Nissi, Ritva; Pihlajaniemi, Taina; Kivirikko, Kari I.
CS Collagen Research Unit, Biocenter and Department of Medical Biochemistry, University of Oulu, Oulu, FIN-90220, Finland
SO EMBO Journal (1997), 16(22), 6702-6712
CODEN: EMJODG; ISSN: 0261-4189
PB Oxford University Press
DT Journal
LA English
AB Prolyl 4-hydroxylase, the key enzyme of collagen synthesis, is an $\alpha 2\beta 2$ tetramer, the β subunit of which is protein disulfide isomerase (PDI). Coexpression of the human α subunit and PDI in Pichia produced trace amts. of an active tetramer. A much higher, although still low, assembly level was obtained using a Saccharomyces pre-pro sequence in PDI. Coexpression with human type III procollagen unexpectedly increased the assembly level 10-fold, with no increase in the total amts. of the subunits. The recombinant enzyme was active not only in Pichia exts. but also inside the yeast cell, indicating that Pichia must have a system for transporting all the cosubstrates needed by the enzyme into the lumen of the endoplasmic reticulum. The 4-hydroxyproline-containing procollagen polypeptide chains were of full length and formed mols. with stable triple helixes even though Pichia probably has no Hsp47-like protein. The data indicate that collagen synthesis in Pichia, and probably also in other cells, involves a highly unusual control mechanism, in that production of a stable prolyl 4-hydroxylase requires collagen expression while assembly of a stable collagen requires enzyme expression. This Pichia system seems ideal for the high-level production of various recombinant collagens for numerous scientific and medical purposes.
RE.CNT 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L2 ANSWER 5 OF 8 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
AN 2004176576 EMBASE
TI The application of recombinant human collagen in tissue engineering.
AU Yang C.; Hillas P.J.; Baez J.A.; Nokelainen M.; Balan J.; Tang J.; Spiro R.; Polarek J.W.
CS Dr. J.W. Polarek, FibroGen Inc., 225 Gateway Blvd, South San Francisco, CA 94080, United States. jpolarek@Fibrogen.com
SO BioDrugs, (2004) 18/2 (103-119).
Refs: 183
ISSN: 1173-8804 CODEN: BIDRF4
CY New Zealand
DT Journal; General Review
FS 027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
LA English
SL English
AB Collagen is the main structural protein in vertebrates. It plays an essential role in providing a scaffold for cellular support and thereby affecting cell attachment, migration, proliferation, differentiation, and survival. As such, it also plays an important role in numerous approaches to the engineering of human tissues for medical applications related to tissue, bone, and skin repair and reconstruction. Currently, the collagen used in tissue engineering applications is derived from animal tissues, creating concerns related to the quality, purity, and predictability of

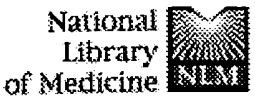
its performance. It also carries the risk of transmission of infectious agents and precipitating immunological reactions. The recent development of recombinant sources of human collagen provides a reliable, predictable and chemically defined source of purified human collagens that is free of animal components. The triple-helical collagens made by recombinant technology have the same amino acid sequence as human tissue-derived collagen. Furthermore, by achieving the equivalent extent of proline hydroxylation via **coexpression** of genes encoding **prolyl hydroxylase** with the **collagen** genes, one can produce collagens with a similar degree of stability as naturally occurring material. The recombinant production process of collagen involves the generation of single triple-helical molecules that are then used to construct more complex three-dimensional structures. If one loosely defines tissue engineering as the use of a biocompatible scaffold combined with a biologically active agent (be it a gene or gene construct, growth factor or other biologically active agent) to induce tissue regeneration, then the production of recombinant human collagen enables the engineering of human tissue based on a human matrix or scaffold. Recombinant human collagens are an efficient scaffold for bone repair when combined with a recombinant bone morphogenetic protein in a porous, sponge-like format, and when presented as a membrane, sponge or gel can serve as a basis for the engineering of skin, cartilage and periodontal ligament, depending on the specific requirements of the chosen application.

L2 ANSWER 6 OF 8 MEDLINE on STN
AN 2004153929 MEDLINE
DN PubMed ID: 15046526
TI The application of recombinant human collagen in tissue engineering.
AU Yang Chunlin; Hillas Patrick J; Baez Julio A; Nokelainen Minna; Balan Juliana; Tang James; Spiro Robert; Polarek James W
CS FibroGen Inc., 225 Gateway Boulevard, South San Francisco, CA 94080, USA.
NC AR 45879 (NIAMS)
SO BioDrugs : clinical immunotherapeutics, biopharmaceuticals and gene therapy, (2004) 18 (2) 103-19. Ref: 183
Journal code: 9705305. ISSN: 1173-8804.
CY New Zealand
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 200406
ED Entered STN: 20040330
Last Updated on STN: 20040630
Entered Medline: 20040629
AB Collagen is the main structural protein in vertebrates. It plays an essential role in providing a scaffold for cellular support and thereby affecting cell attachment, migration, proliferation, differentiation, and survival. As such, it also plays an important role in numerous approaches to the engineering of human tissues for medical applications related to tissue, bone, and skin repair and reconstruction. Currently, the collagen used in tissue engineering applications is derived from animal tissues, creating concerns related to the quality, purity, and predictability of its performance. It also carries the risk of transmission of infectious agents and precipitating immunological reactions. The recent development of recombinant sources of human collagen provides a reliable, predictable and chemically defined source of purified human collagens that is free of animal components. The triple-helical collagens made by recombinant technology have the same amino acid sequence as human tissue-derived collagen. Furthermore, by achieving the equivalent extent of proline hydroxylation via **coexpression** of genes encoding **prolyl hydroxylase** with the **collagen** genes, one can produce collagens with a similar degree of stability as naturally occurring

material. The recombinant production process of collagen involves the generation of single triple-helical molecules that are then used to construct more complex three-dimensional structures. If one loosely defines tissue engineering as the use of a biocompatible scaffold combined with a biologically active agent (be it a gene or gene construct, growth factor or other biologically active agent) to induce tissue regeneration, then the production of recombinant human collagen enables the engineering of human tissue based on a human matrix or scaffold. Recombinant human collagens are an efficient scaffold for bone repair when combined with a recombinant bone morphogenetic protein in a porous, sponge-like format, and when presented as a membrane, sponge or gel can serve as a basis for the engineering of skin, cartilage and periodontal ligament, depending on the specific requirements of the chosen application.

L2 ANSWER 7 OF 8 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
AN 2004:389491 SCISEARCH
GA The Genuine Article (R) Number: 813EL
TI The application of recombinant human collagen in tissue engineering
AU Yang C L; Hillas P J; Baez J A; Nokelainen M; Balan J; Tang J; Spiro R; Polarek J W (Reprint)
CS FibroGen Inc, 225 Gateway Blvd, San Francisco, CA 94080 USA (Reprint); FibroGen Inc, San Francisco, CA 94080 USA
CYA USA
SO BIODRUGS, (16 APR 2004) Vol. 18, No. 2, pp. 103-119.
Publisher: ADIS INTERNATIONAL LTD, 41 CENTORIAN DR, PRIVATE BAG 65901, MAIRANGI BAY, AUCKLAND 10, NEW ZEALAND.
ISSN: 1173-8804.
DT General Review; Journal
LA English
REC Reference Count: 177
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Collagen is the main structural protein in vertebrates. It plays an essential role in providing a scaffold for cellular support and thereby affecting cell attachment, migration, proliferation, differentiation, and survival. As such, it also plays an important role in numerous approaches to the engineering of human tissues for medical applications related to tissue, bone, and skin repair and reconstruction. Currently, the collagen used in tissue engineering applications is derived from animal tissues, creating concerns related to the quality, purity, and predictability of its performance. It also carries the risk of transmission of infectious agents and precipitating immunological reactions. The recent development of recombinant sources of human collagen provides a reliable, predictable and chemically defined source of purified human collagens that is free of animal components. The triple-helical collagens made by recombinant technology have the same amino acid sequence as human tissue-derived collagen. Furthermore, by achieving the equivalent extent of proline hydroxylation via **coexpression** of genes encoding **prolyl hydroxylase** with the **collagen** genes, one can produce collagens with a similar degree of stability as naturally occurring material. The recombinant production process of collagen involves the generation of single triple-helical molecules that are then used to construct more complex three-dimensional structures. If one loosely defines tissue engineering as the use of a biocompatible scaffold combined with a biologically active agent (be it a gene or gene construct, growth factor or other biologically active agent) to induce tissue regeneration, then the production of recombinant human collagen enables the engineering of human tissue based on a human matrix or scaffold. Recombinant human collagens are an efficient scaffold for bone repair when combined with a recombinant bone morphogenetic protein in a porous, sponge-like format, and when presented as a membrane, sponge or gel can serve as a basis for the engineering of skin, cartilage and periodontal ligament, depending on the specific requirements of the chosen application.

L2 ANSWER 8 OF 8 TOXCENTER COPYRIGHT 2004 ACS on STN
AN 2004:119224 TOXCENTER
CP Copyright 2004 ACS
TI The application of recombinant human collagen in tissue engineering
AU Yang, Chunlin; Hillas, Patrick J.; Baez, Julio A.; Nokelainen, Minna; Balan, Juliana; Tang, James; Spiro, Robert; Polarek, James W.
CS FibroGen Inc., San Francisco, CA, USA.
SO BioDrugs, (2004) Vol. 18, No. 2, pp. 103-119.
CODEN: BIDRF4. ISSN: 1173-8804.
CY UNITED STATES
DT Journal
FS CAPLUS
OS CAPLUS 2004:407974
LA English
ED Entered STN: 20040525
Last Updated on STN: 20040525
AB Collagen is the main structural protein in vertebrates. It plays an essential role in providing a scaffold for cellular support and thereby affecting cell attachment, migration, proliferation, differentiation, and survival. As such, it also plays an important role in numerous approaches to the engineering of human tissues for medical applications related to tissue, bone, and skin repair and reconstruction. Currently, the collagen used in tissue engineering applications is derived from animal tissues, creating concerns related to the quality, purity, and predictability of its performance. It also carries the risk of transmission of infectious agents and precipitating immunol. reactions. The recent development of recombinant sources of human collagen provides a reliable, predictable and chemical defined source of purified human collagens that is free of animal components. The triple-helical collagens made by recombinant technol. have the same amino acid sequence as human tissue-derived collagen. Furthermore, by achieving the equivalent extent of proline hydroxylation via **coexpression** of genes encoding **prolyl hydroxylase** with the **collagen** genes, one can produce collagens with a similar degree of stability as naturally occurring material. The recombinant production process of collagen involves the generation of single triple-helical mols. that are then used to construct more complex three-dimensional structures. If one loosely defines tissue engineering as the use of a biocompatible scaffold combined with a biol. active agent (be it a gene or gene construct, growth factor or other biol. active agent) to induce tissue regeneration, then the production of recombinant human collagen enables the engineering of human tissue based on a human matrix or scaffold. Recombinant human collagens are an efficient scaffold for bone repair when combined with a recombinant bone morphogenetic protein in a porous, sponge-like format, and when presented as a membrane, sponge or gel can serve as a basis for the engineering of skin, cartilage and periodontal, ligament, depending on the specific requirements of the chosen application.



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